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(54) Title: SALMONELLA POLYNUCLEOTIDE SEQUENCE

(57) Abstract

DNA comprising a sequence characteristic of certain serotypes of the genus Salmonella is provided and used as polymerase chain reaction and hybridization targets for the identification of said serotypes. The DNA, in recombinant form, e.g. as plasmids, is used to transform suitable host cells to make them capable of expressing amino acid sequences characteristic of said serotypes. Test kits are provided comprising probes targeted at the characteristic sequences and amino acid sequences expressed by the transformants may also be used in immunological test kits for the serotypes.

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+ DESIGNATIONS OF "SU"

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SALMONELLA POLYNUCLEOTIDE SEQUENCE

This invention relates to polynucleotides (DNA) comprising a sequence characteristic of certain serotypes of the genus Salmonella; to the use of sequences comprising the characteristic sequence as polymerase chain reaction and hybridization targets for the identification of said serotypes and to test kits for this; to the use of polynucleotides comprising the sequence to transform suitable host cells to make them capable of expressing amino acid sequences characteristic of said strains; to said amino acid sequence when so expressed and kits containing them; and to plasmids and transformed cells containing said polynucleotide sequences.

Organisms of the genus Salmonella, in particular S. enteritidis, S. dublin and S. typhimurium are responsible for infective food poisoning caused by their ingestion in contaminated food. Infection with Salmonella may also occur as a result of contact with contaminated materials. Once ingested, Salmonella is able to establish itself in the gut and multiply rapidly, resulting in the appearance of clinical symptoms several days after the initial ingestion.

It is therefore highly desirable to provide test methods by means of which Salmonella organisms may be detected. In recent years immunological tests have been devised in which specific antibodies, particularly monoclonal antibodies ("MABs"), to specific antigens are raised and which by exploitation of the antigen - antibody specific binding reaction the presence of the antigen can be detected. Such tests are fast and very specific.

It is known that Salmonella organisms have fimbria like structures on their surface (Duguid; J. P and R. R. Gillies (1958) J. Pathol. Bacteriol. 75:519-520) and published evidence (Clegg, S., and G. F. Gerlach (1987) J. Bacteriol. 169:934-938.) suggests that there are antigenically distinct types of fimbriae, ie. possessing specific epitopes on the fimbrial antigens. The possibility of immunogenic

tests for Salmonella, at least S. enteritidis, based upon these fimbrial antigens has been suggested (MAFF, Central Veterinary Laboratory "Animal Health" (1989):33). Methods of raising MABs to antigens on the surface of micro-organisms such as Salmonella are generally known.

Unfortunately known methods for raising antibodies to Salmonella surface antigens only go part way toward providing an immunological test for Salmonella. The basis of all these tests is to isolate micro-organisms from a sample suspected of harbouring Salmonella, then to grow the micro-organisms in vitro in a suitable culture medium until a quantity of the Salmonella sufficient to detect by such a test is believed to be present in the medium, and then applying the test. A problem occurs in that although Salmonella micro-organisms produce their fimbrial antigen when they grow in vivo, eg. in the gut, in animal tissues or fluids, in food products and in some natural environments, many of the fimbrial antigens are not produced when they are grown in vitro.

The present inventors have determined the polynucleotide sequence responsible for producing a characteristic fimbrial antigen, Salmonella enteritidis fimbrial antigen (SEFA). SEFA has an amino acid sequence forming an epitope on the fimbria 'in vivo' which is specifically found encoded by the DNA of the species S. enteritidis and some strains of the species S. dublin and S. Moscow but which is apparently absent in virtually all other serotypes. The identification and recognition of the significance of this sequence provides the basis for a number of determinative tests for the presence of the particular organisms or DNA/RNA derived from them and provides a method for production of transformed organisms capable of expressing SEFA or epitopic parts of SEFA.

The amino acid sequence of SEFA is provided below; it is of course to be expected that allelic variation will occur in some organisms.

AMINO ACID SEQUENCE OF SALMONELLA ENTERITIDIS FIMBRIAL ANTIGEN

M L I V D F W R F C N M R K S A S A V A V L A L I A C G S A H A A G F
V G N K A E V Q A A V T I A A Q N T T S A N W S Q D P G F T G P A V A
A G Q K V G T L S I T A T G P H N S V S I A G K G A S V S G G V A T V
P F V D G Q G Q P V F R G R I Q G A N I N D Q A N T G I D G L A G W R
V A S S Q E T L N V P V T T F G K S T L P A G T F T A T F Y V Q Q Y Q
N

The codes above are standard codes, read amino-terminal to carboxy -terminal, left to right, M to N, according to the following key:

Amino acid

Alanine	A	Lysine	K	Arginine	R
Methionine	M	Asparagine	N	Phenylalanine	F
Aspartic acid	D	Proline	P	Cysteine	C
Pyroglutamyl	*E	Glutamic acid	E	Serine	S
Glutamine	Q	Threonine	T	Glycine	G
Tryptophan	W	Histidine	H	Tyrosine	Y
Isoleucine	I	Valine	V	Leucine	L

Thus in its broadest form the present invention relates to DNA which forms all or part of the coding sequence for the SEFA sequence above or to allelic variants of that sequence, which carry the codons for its characteristic epitopes.

A first preferred aspect of the present invention provides recombinant DNA comprising the sequences I and II:

Sequence I

```
5'-  G CTCAGAATAC AACATCAGCC  AACTGGAGTC  AGGAT  -3'
3'-  C GAGTCTTATG TTGTAGTCGG  TTGACCTCAG  TCCTA  -5'
          230        240         250
```

Sequence II

```
5'-  CCTGG CTTTACAGGG CCTGCTGTTG CTGCTGGTCA GAAAGTTGGT
3'-  GGACC GAAATGTCCC GGACGACAAC GACGACCAGT CTTTCAACCA
       260        270        280        290        300

ACTCTCAGCA TTACTGCTAC TGGTCCACAT AACTCAGTAT CTATTGCAGG TAAAGGGGCT
TGAGAGTCGT AATGACGATG ACCAGGTGTA TTGAGTCATA GATAACGTCC ATTTCCCCGA
       310        320        330        340        350        360

TCGGTATCTG GTGGTGTAGC CACTGTCCCG TTCGTTGATG GACAAGGACA GCCTGTTTT -3'
AGCCATAGAC CACCACATCG GTGACAGGGC AAGCAACTAC CTGTTCCTGT CGGACAAAA -5'
       370        380        390        400        410
```

sequences degenerately equivalent thereto, or sequences encoding for allelic variants of the part of SEFA for which the sequences I and II encode.

The numerals below each ten base pair sequence in sequence I and II above are those designating the position of the individual base pairs in a larger characteristic sequence that comprises the entire SEFA antigen coding polynucleotide sequence.

By 'degenerately equivalent' is meant that substitute codons are present, these being codons which though they differ in their nucleotide base sequence from the codons identified in sequences I and II above, still code for the same amino acid, as will be understood by a man skilled in the art.

Preferred recombinant DNA of the invention, comprising sequences I and II, is that comprising sequences III and IV:

Sequence III

```
5'- ATGCTAAT AGTTGATTTT TGGAGATTTT GTAATATGCG TAAATCAGCA
3'- TACGATTA TCAACTAAAA ACCTCTAAAA CATTATACGC ATTTAGTCGT
          80         90        100        110        120

TCTGCAGTAG CAGTTCTTGC TTTAATTGCA TGTGGCAGTG CCCACGCAGC TGGCTTTGTT
AGACGTCATC GTCAAGAACG AAATTAACGT ACACCGTCAC GGGTGCGTCG ACCGAAACAA
       130        140        150        160        170        180

GGTAACAAAG CAGAGGTTCA GGCAGCGGTT ACTATTGCAG CTCAGAATAC AACATCAGCC
CCATTGTTTC GTCTCCAAGT CCGTCGCCAA TGATAACGTC GAGTCTTATG TTGTAGTCGG
       190        200        210        220        230        240

AACTGGAGTC AGGAT -3'
TTGACCTCAG TCCTA -5'
       250
```

Sequence IV

```
5'-  CCTGG CTTTACAGGG CCTGCTGTTG CTGCTGGTCA GAAAGTTGGT
3'-  GGACC GAAATGTCCC GGACGACAAC GACGACCAGT CTTTCAACCA
       260        270        280        290        300
```

ACTCTCAGCA TTACTGCTAC TGGTCCACAT AACTCAGTAT CTATTGCAGG TAAAGGGGCT
TGAGAGTCGT AATGACGATG ACCAGGTGTA TTGAGTCATA GATAACGTCC ATTTCCCCGA
310 320 330 340 350 360

TCGGTATCTG GTGGTGTAGC CACTGTCCCG TTCGTTGATG GACAAGGACA GCCTGTTTTC
AGCCATAGAC CACCACATCG GTGACAGGGC AAGCAACTAC CTGTTCCTGT CGGACAAAAG
370 380 390 400 410 420

CGTGGGCGTA TTCAGGGAGC CAATATTAAT GACCAAGCAA ATACTGGAAT TGACGGGCTT
GCACCCGCAT AAGTCCCTCG GTTATAATTA CTGGTTCGTT TATGACCTTA ACTGCCCGAA
430 440 450 460 470 480

GCAGGTTGGC GAGTTGCCAG CTCTCAAGAA ACGCTAAATG TCCCTGTCAC AACCTTTGGT
CGTCCAACCG CTCAACGGTC GAGAGTTCTT TGCGATTTAC AGGGACAGTG TTGGAAACCA
490 500 510 520 530 540

AAATCGACCC TGCCAGCAGG TACTTTCACT GCGACCTTCT ACGTTCAGCA GTATCAAAAC -3'
TTTAGCTGGG ACGGTCGTCC ATGAAAGTGA CGCTGGAAGA TGCAAGTCGT CATAGTTTTG -5'
550 560 570 580 590 600

sequences degenerately equivalent thereto or sequences which encode for allelic variants of SEFA.

The significance of sequences III and IV is that when they run contiguously together, such that the -3' end of the top strand of sequence III is immediately followed by the top strand 5'- end of sequence IV, they consist of the polynucleotide sequence that encodes the amino acid sequence for SEFA (said upper strand).

Thus polynucleotide sequence encoding SEFA is on the upper strand as shown above beginning ATGCTAATAG on III and ending GTATCAAAAC on

IV. Further sequences which comprise suitable flanking sequences for control of amino acid sequence expression may be produced by genetic engineering techniques from this continuous sequence.

The invention further provides recombinant DNA comprising sequence III and IV, in the form of that comprising sequences V and VI:

Sequence V

```
5'- GATCCTTGTT TTTTTTCTTA AATTTTTAAA ATGGCGTGAG TATATTAGCA TCCGCACAGA
3'- CTAGGAACAA AAAAAAGAAT TTAAAAATTT TACCGCACTC ATATAATCGT AGGCGTGTCT
            10         20         30         40         50         60

    TAAATTGTGC GAATGCTAAT AGTTGATTTT TGGAGATTTT GTAATATGCG TAAATCAGCA
    ATTTAACACG CTTACGATTA TCAACTAAAA ACCTCTAAAA CATTATACGC ATTTAGTCGT
            70         80         90        100        110        120

    TCTGCAGTAG CAGTTCTTGC TTTAATTGCA TGTGGCAGTG CCCACGCAGC TGGCTTTGTT
    AGACGTCATC GTCAAGAACG AAATTAACGT ACACCGTCAC GGGTGCGTCG ACCGAAACAA
           130        140        150        160        170        180

    GGTAACAAAG CAGAGGTTCA GGCAGCGGTT ACTATTGCAG CTCAGAATAC AACATCAGCC
    CCATTGTTTC GTCTCCAAGT CCGTCGCCAA TGATAACGTC GAGTCTTATG TTGTAGTCGG
           190        200        210        220        230        240

    AACTGGAGTC AGGAT -3'
    TTGACCTCAG TCCTA -5'
           250
```

Sequence VI

```
                   5'- CCTGG CTTTACAGGG CCTGCTGTTG CTGCTGGTCA GAAAGTTGGT
                   3'- GGACC GAAATGTCCC GGACGACAAC GACGACCAGT CTTTCAACCA
                         260        270        280        290        300

ACTCTCAGCA TTACTGCTAC TGGTCCACAT AACTCAGTAT CTATTGCAGG TAAAGGGGCT
TGAGAGTCGT AATGACGATG ACCAGGTGTA TTGAGTCATA GATAACGTCC ATTTCCCCGA
       310        320        330        340        350        360

TCGGTATCTG GTGGTGTAGC CACTGTCCCG TTCGTTGATG GACAAGGACA GCCTGTTTTC
AGCCATAGAC CACCACATCG GTGACAGGGC AAGCAACTAC CTGTTCCTGT CGGACAAAAG
       370        380        390        400        410        420

CGTGGGCGTA TTCAGGGAGC CAATATTAAT GACCAAGCAA ATACTGGAAT TGACGGGCTT
GCACCCGCAT AAGTCCCTCG GTTATAATTA CTGGTTCGTT TATGACCTTA ACTGCCCGAA
       430        440        450        460        470        480

GCAGGTTGGC GAGTTGCCAG CTCTCAAGAA ACGCTAAATG TCCCTGTCAC AACCTTTGGT
CGTCCAACCG CTCAACGGTC GAGAGTTCTT TGCGATTTAC AGGGACAGTG TTGGAAACCA
       490        500        510        520        530        540

AAATCGACCC TGCCAGCAGG TACTTTCACT GCGACCTTCT ACGTTCAGCA GTATCAAAAC
TTTAGCTGGG ACGGTCGTCC ATGAAAGTGA CGCTGGAAGA TGCAAGTCGT CATAGTTTTG
       550        560        570        580        590        600

TAATTTAATT TAAACTTTAT AAATGCCCTC AATATGAGCG AGTTTGGATA ATTTTATTAT
ATTAAATTAA ATTTGAAATA TTTACGGGAG TTATACTCGC TCAAACCTAT TAAAATAATA
       610        620        630        640        650        660
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```
TTTAAAAATA TCTATTTTGA ATAGATAGGT TTTATGCTTC CATGCAAAAA CTTAAAGAGG
AAATTTTTAT AGATAAAACT TATCTATCCA AAATACGAAG GTACGTTTTT GAATTTCTCC
       670        680        690        700        710        720

GATTATGTAT ATTTTGAATA AATTTATACG TAGAACTGTT ATCTTTTTCC TTTTTTTTGC
CTAATACATA TAAAACTTAT TTAAATATGC ATCTTGACAA TAGAAAAAGG AAAAAAAACG
       730        740        750        760        770        780

TACCTTCCAA TTGCTTCTTC GGAAAGTAAA AAAATTGAGC AACCATTATT AACACAAAAA
ATGGAAGGTT AACGAAGAAG CCTTTCATTT TTTTAACTCG TTGGTAATAA TTGTGTTTTT
       790        800        810        820        830        840

TATTATGGCC TAAGATTGGG CACTACACGT GTTATTTATA AAGAAGATGC TCCATCAACA
ATAATACCGG ATTCTAACCC GTGATGTGCA CAATAAATAT TTCTTCTACG AGGTAGTTGT
       850        860        870        880        890        900

AGTTTTTGGA TTATGAATGA AAAAGAATAT CCAATCCTTG TTCAAACTCA AGTATATAAT
TCAAAAACCT AATACTTACT TTTTCTTATA GGTTAGGAAC AAGTTTGAGT TCATATATTA
       910        920        930        940        950        960

GATGATAAAT CATCAAAAGC TCCATTTATT GTAACACCAC CTATTTTGAA AGTTGAAAGT
CTACTATTTA GTAGTTTTCG AGGTAAATAA CATTGTGGTG GATAAAACTT TCAACTTTCA
       970        980        990       1000       1010       1020

AATGCGCGAA CAAGATTGAA GGTAATACCA ACAAGTAATC TATTCAATAA AAATGAGGAG
TTACGCGCTT GTTCTAACTT CCATTATGGT TGTTCATTAG ATAAGTTATT TTTACTCCTC
      1030       1040       1050       1060       1070       1080
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TCTTTGTATT GGTTGTGTGT AAAAGGAGTC CCACCACTAA ATGATAATGA AAGCAATAAT
AGAAACATAA CCAACACACA TTTTCCTCAG GGTGGTGATT TACTATTACT TTCGTTATTA
      1090       1100       1110       1120       1130       1140

AAAAACAACA TAACTACGAA TCTTAATGTG AATGTGGTTA CGAATAGTTG TATTAAATTA
TTTTTGTTGT ATTGATGCTT AGAATTACAC TTACACCAAT GCTTATCAAC ATAATTTAAT
      1150       1160       1170       1180       1190       1200

ATTTATAGGC CTAAAACTAT AGACTTAACG ACAATGGAGA TTGCAGATAA ATTAAAGTTA
TAAATATCCG GATTTTGATA TCTGAATTGC TGTTACCTCT AACGTCTATT TAATTTCAAT
      1210       1220       1230       1240       1250       1260

GAGAGAAAAG GAAATAGTAT AGTTATAAAG AATCCAACAT CATCATATGT GAATATTGCA
CTCTCTTTTC CTTTATCATA TCAATATTTC TTAGGTTGTA GTAGTATACA CTTATAACGT
      1270       1280       1290       1300       1310       1320

AATATTAAAT CTGGTAATTT AAGTTTTAAT ATTCCAAATG GATATATTGA GCCATTTGGA
TTATAATTTA GACCATTAAA TTCAAAATTA TAAGGTTTAC CTATATAACT CGGTAAACCT
      1330       1340       1350       1360       1370       1380

TATGCTCAAT TACCTGGTGG AGTACATAGT AAAATAACTT TGACTATTTT GGATGATAAC
ATACGAGTTA ATGGACCACC TCATGTATCA TTTTATTGAA ACTGATAAAA CCTACTATTG
      1390       1400       1410       1420       1430       1440

GGCGCTGAAA TTATAAGAGA ATTATTAGTT TAAGGTGTAA AACAAATGAA GAAAACCACA
CCGCGACTTT AATATTCTCT TAATAATCAA ATTCCACATT TTGTTTACTT CTTTTGGTGT
      1450       1460       1470       1480       1490       1500
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ATTACTCTAT TTGTTTTAAC CAGTGTATTT CACTCTGGAA ATGTTTTCTC CAGACAATAT
TAATGAGATA AACAAAATTG GTCACATAAA GTGAGACCTT TACAAAAGAG GTCTGTTATA
      1510       1520       1530       1540       1550       1560

AATTTCGACT ATGGAAGTTT GAGTCTTCTC CCGGTGAGAA TGCATCTTTT CTAAGTGTTG
TTAAAGCTGA TACCTTCAAA CTCAGAAGAG GGCCACTCTT ACGTAGAAAA GATTCACAAC
      1570       1580       1590       1600       1610       1620

AAACGCTTCC CTGGTAATTA TGTTGTTGAT GTATATTTGA ATAATCAGTT AAAAGAAACT
TTTGCGAAGG GACCATTAAT ACAACAACTA CATATAAACT TATTAGTCAA TTTTCTTTGA
      1630       1640       1650       1660       1670       1680

ACTGAGTTGT ATTTCAAATC AATGACTCAG ACTCTAGAAC CATGCTTAAC AAAAGAAAAA
TGACTCAACA TAAAGTTTAG TTACTGAGTC TGAGATCTTG GTACGAATTG TTTTCTTTTT
      1690       1700       1710       1720       1730       1740

CTTATAAAGT ATGGGATCGC CATCCAGGAG CTTCATGGGT TGCAGTTTGA TAATGAACAA
GAATATTTCA TACCCTAGCG GTAGGTCCTC GAAGTACCCA ACGTCAAACT ATTACTTGTT
      1750       1760       1770       1780       1790       1800

TGCGTTCTCT TAGAGCATTC TCCTCTTTAA ATATACTTAT AACGCGGCTA ACCAAAGTTT
ACGCAAGAGA ATCTCGTAAG AGGAGAAATT TATATGAATA TTGCGCCGAT TGGTTTCAAA
      1810       1820       1830       1840       1850       1860

GCTTTTAAAT GCACCATCTA AAATTCTATC TCCAATAGAC AGTGAAATTG CTGATGAAAA
CGAAAATTTA CGTGGTAGAT TTTAAGATAG AGGTTATCTG TCACTTTAAC GACTACTTTT
      1870       1880       1890       1900       1910       1920
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TATCTGGGAT GATGGCATTA ACGCTTTTCT TTTAAATTAC AGAGCTTAAT TATTTGCATT
ATAGACCCTA CTACCGTAAT TGCGAAAAGA AAATTTAATG TCTCGAATTA ATAAACGTAA
      1930       1940       1950       1960       1970       1980

CTAAGGTTGG AGGAGAGAGA TTCATACTTT GGTCAAATTC AACCTTGGTT TTAATTTTGG
GATTCCAACC TCCTCTCTCT AAGTATGAAA CCAGTTTAAG TTGGAACCAA AATTAAAACC
      1990       2000       2010       2020       2030       2040

TCCCTGGCGG CTAAGGAATC TATCATCTTG GCAAAACTTG TCAAGCGAAA AAAAATTTGA
AGGGACCGCC GATTCCTTAG ATAGTAGAAC AGTTTTGAAC AGTTCGCTTT TTTTTAAACT
      2050       2060       2070       2080       2090       2100

ATCAGCATAT ATTTATGCTG AGCGAGGTTT AAAAAAAATA AAGAGCAAAC TAACAGTTGG
TAGTCGTATA TAAATACGAC TCGCTCCAAA TTTTTTTTAT TTCTCGTTTG ATTGTCAACC
      2110       2120       2130       2140       2150       2160

GGACAAATAT ACCAGTGCAG ATTTATTCGA TAGCGTACCA TTTAGAGGCT TTTCTTTAAA
CCTGTTTATA TGGTCACGTC TAAATAAGCT ATCGCATGGT AAATCTCCGA AAAGAAATTT
      2170       2180       2190       2200       2210       2220

TAAAGATGAA AGTATGATAC CTTTCTCACA GAGAACATAT TATCCAACAA TACGTGGTAT
ATTTCTACTT TCATACTATG GAAAGAGTGT CTCTTGTATA ATAGGTTGTT ATGCACCATA
      2230       2240       2250       2260       2270       2280

TGCGAAAACC AATGCGACTG TAGAAGTAAG ACAAAATGGA TACTTGATAT ATTCTACTTC
ACGCTTTTGG TTACGCTGAC ATCTTCATTC TGTTTTACCT ATGAACTATA TAAGATGAAG
      2290       2300       2310       2320       2330       2340

AGTCCCCCCC GGGCAATTCG AGATAGGTAG AGAACAAATT GCTGATC -3'
TCAGGGGGGG CCCGTTAAGC TCTATCCATC TCTTGTTTAA CGACTAG -5'
      2350       2360       2370       2380
```

or sequences degeneratively equivalent thereto.

For the purposes of expressing SEFA polypeptide or epitopic parts thereof the paired sequences I and II; III and IV; or V and VI run contiguously with each other without intervening base pairs between the two, in each case. These contiguous sequences are designated sequence VII, VIII and IX respectively.

For the purpose of expressing SEFA it will be realised by the skilled man that all the sequences above may comprise degenerate codons instead of those listed above. It is not envisaged that such use will necessarily provide any advantage as preparation would be probably be more lengthy, but some transformed microorganisms may express SEFA more readily with certain codons in degenerate form suited to them.

The present invention provides novel recombinant plasmids, comprising the recombinant DNA comprising either paired sequences selected from I and II, III and IV, or V and VI or the contiguous sequences VII, VIII and IX, the degenerative or allelic equivalents of any of these; said plasmids being capable of expressing polypeptides characteristic of SEFA when used to transform suitable microorganisms.

These recombinant plasmids may then be used to transform a host, such as E coli or yeast, whereby use of cloning and selection methods provides clones which contain the particular sequence or suitably flanked antigen encoding portion having expression enabling sequences with it. Convenient tools for the selection of these clones are the aforementioned sequences themselves as modified in known ways to provide probes, ie. by radiolabelling. Such probe sequences are readily provided by use of the polymerase chain reaction on native SEFA sequence template or by DNA synthesizer techniques; radiolabelling being achieved using standard techiques to tag on ^{32}P.

Preferred microorganisms for transformation are E. coli and yeasts; a particularly preferred microorganism being E. coli DH5alpha. Thus preferred plasmids will be those known to the man skilled in the art as suitable for transforming such organisms. Particularly preferred plasmids are accordingly pBR322, pACYC184 and, most preferred, pUC18.

The polynucleotides sequences above may be combined with any of these known plasmids for the purposes of providing the novel plasmids of the invention. Particularly preferred will be plasmids into which polynucleotides consisting of the contiguous sequences VIII or IX have been inserted as these will be readily provided from cultured S. enteritidis or S. dublin by use of restriction endonucleases and encode for the entire SEFA amino acid sequence. In this respect use of antibodies targeted for SEFA allows facile recognition of transformed organisms which is particularly useful for selecting expressing organisms from a background population. Such antibodies are the subject of copending MAFF patent application (PCT GB 91-----, our reference P0958) of inventor C J Thorns).(See Tables I and II).

For example, the contiguous sequence IX may be blunt-ended using Klenow polymerase infilling and then ligated into a plasmid such as pUC18. Alternatively total genomic DNA is extracted from S. enteritidis or a strain of S. dublin possessing said fimbrial antigen, as determined using the monoclonal antibodies and techniques disclosed in the applicants copending application referred to above, and then partially digested with SauIIIA restriction endonuclease to leave large fragments, some of which contain the sequences referred to above, which are then ligated into the plasmid vectors above.

The vectors of the present invention have further utility in so far as the contiguous sequences VII, VIII and IX all comprise a single BamH1 restriction endonuclease recognition site into which foreign peptide encoding DNA may be ligated by which it is sited within the reading frame of the transformant transcription system. This site is

at the junction between the two sequences that make up the contiguous sequence; that occuring between base pair 255 and 256 in the numbering system applied at the bottom of each 10 base pairs above. Thus the present invention provides plasmids and transformants comprising the sequences I and II, or III and IV, or V and VI, or their degenerative or allelic equivalents, which have been augmented with further sequences. The invention provides a method for preparing these plasmid and transformants which inserts the further sequences into plasmids comprising the contiguous sequences VII, VIII or IX the at that BamH1 site.

Such augmented transformants are potentially capable of expression of mixed epitopic polypeptides comprising epitopes of SEFA together with further 'foreign' peptides. This opens the way to recombinantly produced peptides that are not easily expressed by other means. The fact that SEFA is a polypeptide that is passed to the exterior of the Salmonella cell of advantage in the recovery of such expressed polypeptides. The 'foreign' peptides may be further SEFA epitopes.

Thus the invention also provides micro-organisms, eg E.coli or yeasts, which have been transformed by insertion of one or more of the aforementioned sequences eg, by use of said plasmids.

Use of the micro-organisms provided by the invention gives a method of expression of the antigenic amino acid sequence SEFA referred to above and epitopic parts thereof which might be used as antigenic activity, that is having the ability to evoke production of antibodies in animal bodies.

In addition to use of the transformant expressed SEFA or epitopic parts thereof for immunological test purposes and kits for such, the recognition of the significance of the DNA sequences defined above provides methods of determination of DNA or RNA as being derived from the S. enteritidis or S. dublin serotypes in other, DNA/RNA based, tests.

TABLE I

264 Salmonella strains examined with monoclonal antibody MAB69/25

Serogroup	Serotype (No. strains tested)	Serogroup	Serotype (No. strains tested)
B	S. agama (1)	D1	S. gallinarium (44)
	S. agona (1)		S. moscow (1)
	S. bredeney (1)		S. ouakam (1)
	S. derby (1)		S. panama (1)
	S. heidelberg (1)		S. pullorum (3)
	S. indiana (1)		S. wangata (1)
	S. reading (1)	E1	S. anatum (1)
	S. schwarzengrund (1)		S. give (1)
	S. stanley (1)		S. lexington (1)
	S. typhimurium (64)		S. london (1)
C1	S. bareilly (1)		S. meleagridis (1)
	S. infantis (1)		S. nchanga (1)
	S. lille (1)		S. orion (1)
	S. livingstone (1)	E2	S. binza (1)
	S. mbandaka (1)		S. drypool (1)
	S. montevideo (1)		S. manila (1)
	S. ohio (1)		S. newington (1)
	S. oranienburg (1)	E4	S. taksony (1)
	S. oslo (1)		S. senftenberg (1)
	S. thompson (1)	F	S. aberdeen (1)
	S. virchow (1)	G1	S. havana (1)
C2	S. goldcoast (1)		S. worthington (1)
	S. hadar (1)	G2	S. ajiobo (1)
	S. newport (1)		S. kedougou (1)
C3	S. albany (1)	K	S. cerro (1)
	S. kentucky (2)	N	S. urbana (1)
	S. tado (1)	O	S. adelaide (1)
D1	S. berta (1)		S. ealing (1)
	S. canastel (1)	R	S. johannesburg (1)
	S. dublin (36)	S	S. offa (1)
	S. durban (1)	T	S. gera (1)
	S. enteritidis (58)		

TABLE II

Direct binding of MAB 69/25 to Salmonella strains

Serotype		Number Examined	Monoclonal antibody MAB 69/25 %bound
S. enteritidis	PT 1	2	56[a] (48-64)[b]
S. enteritidis	PT 4	22	57 (14-100)
S. enteritidis	PT 4 plasmid minus	6	57 (49-65)
S. enteritidis	PT 5	1	83
S. enteritidis	PT 6	1	57
S. enteritidis	PT 7	1	89 (85-93)
S. enteritidis	PT 8	12	53 (15-90)
S. enteritidis	PT 9	4	20 (17-23)
S. enteritidis	PT 11	7	50 (23-77)
S. enteritidis	PT 30	1	15
S. enteritidis	untypable	1	41
S. dublin		12	25 (9-40)
S. dublin		24	0
S. moscow		1	9
Other Salmonella strains[c]		169	0

[a] Mean percentage of antibody binding relative to binding to high control (see text)

[b] Range of binding

[c] Serotypes listed in Table I

PT = Phage type

The present invention further provides methods for determining the presence of microorganisms having DNA or RNA polynucleotide sequence encoding for SEFA or an epitopic part thereof, or such DNA or RNA itself, comprising:

(a) providing a sample suspected of containing said encoding polynucleotide sequence;

(b) determining the presence of said sequence by monitoring hybridization of SEFA targeted polynucleotide probes to it.

Such hybridization technique is carried out by methods that are now conventional in the art, using probes which are comprised of sequences complementary to a significant part of the target sequence and using temperature conditions suitable to achieved a desired stringency dependent on the degree of match of the probe to the target.

In a preferred form of this method the invention further provides methods for determining the presence of microorganisms having DNA or RNA polynucleotide sequence encoding for SEFA or an epitopic part thereof, or such DNA or RNA itself, comprising:

(a) providing a sample suspected of containing said encoding polynucleotide sequence;

(b) subjecting said sample to conditions under which polynucleotide sequences comprising sequences (I) and (II) are replicated by use of the polymerase chain reaction;

(c) determining the presence of any sequence produced.

Conveniently the sequence produced is detected, in both cases, by use of a hybridization probe suitably specific thereto which comprises any

of the aforementioned sequences, more specifically being one of the sequences in a suitably labelled form eg. being labelled in some way as will be known to a man skilled in the art. Most conveniently the label will incorporate radioactive phophorous (^{32}P). A preferred such method comprises a PCR step (b) which employs primer pairs comprising one primer selected from groups (A) and the other from group(B):

Group A:	Group B:
A1: 5' -GTGCGAATGCTAATAGTTGA- 3'	B1: 5' -AAAACAGGCTGTCCTTGTCCA- 3'
A2: 5' -TGCGTAAATCAGCATCTGCA- 3'	B2: 5' -TTAGCGTTTCTTGAGAGCTGG- 3'
A3: 5' -TCTGCAGTAGCAGTTCTTGC- 3'	B3: 5' -TTTTGATACTGCTGAACGTAG- 3'
A4: 5' -GCTCAGAATACAACATCAGCCAA- 3'	

The primers are numbered A1 to A4 and B1 to B3 for the purposes of identification later in this specification.

Any of the possible pairs selected in this way will identify the characteristic sequences VI, VII or IX sufficiently specifically enough for serotype determination purposes, ie: for determination of a Salmonella as being a SEFA encoding serotype and thus of one of the serotypes listed above.

As will be understood by a man skilled in the art, sequences which will specifically hybridize with sequence (VII) will include sequence (VII) itself, those having 75% or more, preferably 90% or more conformity to that sequence, and sequences comprising either strand of the two complementary sequences of any of these. Thus the step (c) of the method of this aspect of the invention may be carried out using a variety of hybridization probes that combine sufficiently

specifically with the characteristic 'target' sequence comprising sequence (VII). For most purposes the primer sequences selected from those of groups (A) and (B) will be sufficiently specific to give reliable determination of the characteristic sequence, especially if a different 'primer' sequence is used for the probe of step (c) than those used for step (b).

The step (b) is carried out using the enzyme Taq polymerase as is now conventional in the art. The necessary conditions are those as described in EP-A-0201184 or EP-A-0200362 (both Cetus Corpn.) In such reaction, the appropriate primers derived from the sequences act as initiators for synthesis of large quantities of DNA identical to, or substantially identical to the initial double stranded DNA sequence. In this way substantially larger quantities of the DNA sequence may be made from the small quantities which may be available by isolation from the S. enteritidis or S. dublin thus increasing the amount of sequence available to be detected. The mere opresence of increased amount of DNA may be used in this case to signify presence of target sequence.

The genetically transformed organisms of the invention and their use to produce SEFA and SEFA containing sequences of the invention will now be described by way of example only, the examples including use of the detection methods of the invention for confirming presence of transformants:

Example A. Preparation and cloning of S. enteritidis fimbrial antigen genes.

Step A1. Total genomic DNA was extracted from S. enteritidis using the method described in J B Goldberg & D E Ohman, (1984) J Bact 158 1115-1121.

Step A2. The DNA from step A1 was partially digested with SauIIIA restriction endonuclease to yield fragments with an size range between 5 and 10 kb. 2ug of genomic DNA in a Tris-HCl based buffer of pH 7.4 were mixed with 0.25 units of SauIIIA and incubated at 37°C.

Step A3. Cloning vector pUC18 was digested to completion with BamH1, giving compatible cohesive ends with SauIIIA, and was dephosphorylated with calf intestinal phosphatase.

Step A4. S. enteritidis DNA was ligated with vector pUC18 using T4 DNA ligase supplied by Bethesda Research Laboratories Life Technologies Inc. (Cat. No. 5224SB/SC). The supplier's instructions for use in ligation were followed.

Step A5. The recombinant plasmid from step A4 was used to transform commercially available E.coli DH5alpha supplied by Bethesda Labs (see above) as Library Efficiency (RTM) DH5alpha Competant Cells (Cat. No. 8263SA) using the supplier's instructions to produce a genomic library.

Step A6. Transformants were transferred to the surface of HYBOND-C filters by replica plating for Western Blotting. Standard Western Blotting procedures using the S. enteritidis fimbrial antigen specific monoclonal antibody MAB 69/25, derived by standard techniques from hybridoma cells deposited under Accession No.90101101 on 11 October 1990 at the European Collection of Animal Cell Cultures, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wiltshire SP4 0JG, United Kingdom, as described and claimed in copending application No (PCT GB91 ;our ref P0958WOD), were done to identify transformant colonies expressing SEFA and thus containing the aforementioned sequences (VI), (VII) and (IX).

Step A7. The recombinant plasmids from fimbrial antigen positive transformants were extracted and used in confirmatory tests to prove

the insert encoded said fimbrial antigen.

At the end of stage A7 it is possible to probe the DNA of said transformants to show the presence of the sequences and then to analyse said sequence by known sequencing methods.

EXAMPLE B: Presentation of epitopes within the SEFA antigen by insertion of foreign DNA, in frame, into the SEFA encoding sequence.

As stated above, the present invention further provides the prospect of exploitation of the polynucleotide sequences of the present invention having with sequences encoding for desired foreign protein or peptide products to produce transformants having ability to secrete the desired product.

SB10 epitope of *Mycobacterium bovis* secreted antigen, MPB70 (Radford et al, (1990), J. Gen. Micro. 136: 265-272) consists of the amino acid sequence as encoded for below:

Q D P V	encoded amino acid
5'- CAG GAC CCG GTC -3'	coding/master strand
3'- GTC CTG GGC CAG -5'	complimentary strand

Synthetic oligonucleotides encompassing this sequence and providing BamH1 cohesive ends were made using an ABI PCR MATE EP model 391 DNA synthesizer following the manufacturer's methods. The oligonucleotides were as follows:

SB10.1	5'-	GAT CAG GAC CCG GTC GCT	-3'
SB10.2	3'-	TC CTG GGC CAG CGA CTA G	-5'

The two oligonucleotides, SB10.1 and SB10.2 were allowed to anneal to form a double stranded (duplex) molecule by heating to 95°C and then cooling to room temperature over a two hour period. Annealing was assessed by comparing rate of migration of the duplex molecule compared with the rate of migration of the two single oligonucleotides when run through 4% agarose in TBE buffer. A marginal retardation in migration rate was observed and suggested near 100% annealing.

A lambda EMBL library was prepared from S.enteritidis strain 1246 providing a 9 to 23 kilobase library which was probed with the SEFA sequence IX (consisting of sequences V and VI run contiguously). Hybridizing fragments were subcloned into pUC18 and a suitable vector comprising the SEFA antigen gene flanked by adjacent contiguous chromosomal DNA was selected on its ability to transform E.coliDH5 alpha to a SEFA expressing form: all general methods as conventional to the art (see eg. Maniatis).

The pUC18 vector so obtained was digested with BamH1 and agarose gel electrophoresis demonstrated that the DNA was cut once at the unique BamH1 site within the SEFA gene. Cut vector and duplex oligonucleotide (SB10.1 plus SB10.2) were mixed together (1:10 ratio) and ligated using T4 ligase (Life Technologies) using the manufacturers methods. The saturating amounts of duplex oligonucleotide increased rate of insertion and the lack of terminal phosphate groups on the duplex prevented multiple insertion. The ligated construct was designed to be as follows:

	Q	D	Q	D	P	V	A	D	P	amino acid
5'-	CAG	GAT	CAG	GAC	CCG	GTC	GCT	GAT	CCT -3'	coding/master strand
3'-	GTC	CTA	GTC	CTG	GGC	CAG	CGA	CTA	GGA -5'	complimentary strand

The ligated construct lacks the GGATCC BamH1 recognition sequence.

Thus prior to transforming the construct into E.coli DH5 alpha, the ligated DNA was cut with BamH1 to linearise any of the vector which lacked insert. The ligated DNA was then used to transform E.coli using standard procedures.

Recombinants were picked directly into a Polymerase Chain Reaction mixture in which the primers were designed to flank the insertion site to yield a product of 219 base pairs without insert or 237 base pairs with insert. PCR products were sized by gel lectrophoresis and those shown to be 237 base pairs were tested by digestion with BamH1 to ensure loss of the site.

A sample (8ul) was taken from the aqueous phase of the PCR reaction mixture and made 20ul by addition of HPLC grade water, X10 reaction buffer and 5U BamH1. The PCR product was digested for 3 hours at 37°C. Control experiments using the 219 base pair product were performed to demonstrate digestion. The entire reaction mixtures were loaded onto agarose gels and the DNA products resolved; those PCR products shown to be 237 base pairs did not cut with BamH1 giving evidence for insertion of the oligonucleotide duplex.

To confirm the presence of the insert and determine its orientation, PCR experiments were set up in which the primers were SB10.2 and a series of primers from primer group A above (see page 18) toward the proximal (5') end of the SEFA antigen gene. Of twelve recombinants tested, five gave the desired sized product and were, therefore, shown to have the insert in the correct orientation.

To confirm that the insert was encoding the SB10 epitope and was 'in frame' with the SEFA antigen sequence, double stranded DNA sequencing using standard protocols was done on the five positive clones identified above. The primers used were:

5'- TCTGCAGTAGCAGTTCTTGC -3' for the coding strand and

5'- AAAACAGGCTGTCCTTGTCCA -3' for the complimentary strand.

The DNA sequence of both strands across the insert site was established and was as predicted above.

E. coli recombinants harbouring the constructs, designated SEFA::SB10. 1 to 5 were tested immunologically for the production of SEFA. Western blots of whole E. coli cells harbouring each of the SEFA::SB10 constructs demonstrated the presence of a protein of about 15kDal (and a less intense protein band of about 18.5 kDal) when using anti-SEFA polyclonal and anti-SEFA monoclonal antibody 69/25. In control experiments, E. coli recombinants harbouring the vector gave protein bands of 14.5kDal and 18kDal in Western blot experiments using the same antibodies.

This data clearly demonstrates that the SEFA polynucleotide sequence may be modified to express additional amino acids within its primary stricture without the loss of reactivity to one SEFA epitope specific antibody.

The complete sequence of the largest of the sequences of the invention, sequence IX, is given below with the sequences I, II, III, IV, V, VI, VII and VIII being indicated together with the probe sequences from probe groups A and B. These sequences are marked by reference to their 5' and 3' ends: eg. I-5', I-3' etc. The numbering given below each 10 base pairs of the sequences I to VI above being related to their positions in this sequence IX.

Sequence IX

```
    V-5'
5'- GATCCTTGTT TTTTTTCTTA AATTTTTAAA ATGGCGTGAG TATATTAGCA TCCGCACAGA
3'- CTAGGAACAA AAAAAAGAAT TTAAAAATTT TACCGCACTC ATATAATCGT AGGCGTGTCT
             10         20         30         40         50         60

         A1-5' III-5'     A1-3'                       A2-5'
    TAAATTGTGC GAATGCTAAT AGTTGATTTT TGGAGATTTT GTAATATGCG TAAATCAGCA
    ATTTAACACG CTTACGATTA TCAACTAAAA ACCTCTAAAA CATTATACGC ATTTAGTCGT
             70         80         90        100        110        120
    A3-5'
      A2-3'                  A3-3'
    TCTGCAGTAG CAGTTCTTGC TTTAATTGCA TGTGGCAGTG CCCACGCAGC TGGCTTTGTT
    AGACGTCATC GTCAAGAACG AAATTAACGT ACACCGTCAC GGGTGCGTCG ACCGAAACAA
            130        140        150        160        170        180

                                            A4-5'
    GGTAACAAAG CAGAGGTTCA GGCAGCGGTT ACTATTGCAG CTCAGAATAC AACATCAGCC
    CCATTGTTTC GTCTCCAAGT CCGTCGCCAA TGATAACGTC GAGTCTTATG TTGTAGTCGG
            190        200        210        220        230        240
    A4-3'
       I,III and V -3':II,IV and VI-5'
    AACTGGAGTC AGGATCCTGG CTTTACAGGG CCTGCTGTTG CTGCTGGTCA GAAAGTTGGT
    TTGACCTCAG TCCTAGGACC GAAATGTCCC GGACGACAAC GACGACCAGT CTTTCAACCA
            250        260        270        280        290        300
               BamH1 site.

    ACTCTCAGCA TTACTGCTAC TGGTCCACAT AACTCAGTAT CTATTGCAGG TAAAGGGGCT
    TGAGAGTCGT AATGACGATG ACCAGGTGTA TTGAGTCATA GATAACGTCC ATTTCCCCGA
            310        320        330        340        350        360
```

```
                                                            II-3'
TCGGTATCTG GTGGTGTAGC CACTGTCCCG TTCGTTGATG GACAAGGACA GCCTGTTTTC
AGCCATAGAC CACCACATCG GTGACAGGGC AAGCAACTAC CTGTTCCTGT CGGACAAAAG
       370        380        390        400        410        420
                                         B1-3'                B1-5'

CGTGGGCGTA TTCAGGGAGC CAATATTAAT GACCAAGCAA ATACTGGAAT TGACGGGCTT
GCACCCGCAT AAGTCCCTCG GTTATAATTA CTGGTTCGTT TATGACCTTA ACTGCCCGAA
       430        440        450        460        470        480

GCAGGTTGGC GAGTTGCCAG CTCTCAAGAA ACGCTAAATG TCCCTGTCAC AACCTTTGGT
CGTCCAACCG CTCAACGGTC GAGAGTTCTT TGCGATTTAC AGGGACAGTG TTGGAAACCA
       490        500        510        520        530        540
             B2-3'                 B2-5'

                                                            IV-3'
AAATCGACCC TGCCAGCAGG TACTTTCACT GCGACCTTCT ACGTTCAGCA GTATCAAAAC
TTTAGCTGGG ACGGTCGTCC ATGAAAGTGA CGCTGGAAGA TGCAAGTCGT CATAGTTTTG
       550        560        570        580        590        600
                                         B3-3'                B3-5'

TAATTTAATT TAAACTTTAT AAATGCCCTC AATATGAGCG AGTTTGGATA ATTTTATTAT
ATTAAATTAA ATTTGAAATA TTTACGGGAG TTATACTCGC TCAAACCTAT TAAAATAATA
       610        620        630        640        650        660

TTTAAAAATA TCTATTTTGA ATAGATAGGT TTTATGCTTC CATGCAAAAA CTTAAAGAGG
AAATTTTTAT AGATAAAACT TATCTATCCA AAATACGAAG GTACGTTTTT GAATTTCTCC
       670        680        690        700        710        720

GATTATGTAT ATTTTGAATA AATTTATACG TAGAACTGTT ATCTTTTTCC TTTTTTTTGC
CTAATACATA TAAAACTTAT TTAAATATGC ATCTTGACAA TAGAAAAAGG AAAAAAAACG
       730        740        750        760        770        780
```

```
TACCTTCCAA TTGCTTCTTC GGAAAGTAAA AAAATTGAGC AACCATTATT AACACAAAAA
ATGGAAGGTT AACGAAGAAG CCTTTCATTT TTTTAACTCG TTGGTAATAA TTGTGTTTTT
       790        800        810        820        830        840

TATTATGGCC TAAGATTGGG CACTACACGT GTTATTTATA AAGAAGATGC TCCATCAACA
ATAATACCGG ATTCTAACCC GTGATGTGCA CAATAAATAT TTCTTCTACG AGGTAGTTGT
       850        860        870        880        890        900

AGTTTTTGGA TTATGAATGA AAAAGAATAT CCAATCCTTG TTCAAACTCA AGTATATAAT
TCAAAAACCT AATACTTACT TTTTCTTATA GGTTAGGAAC AAGTTTGAGT TCATATATTA
       910        920        930        940        950        960

GATGATAAAT CATCAAAAGC TCCATTTATT GTAACACCAC CTATTTTGAA AGTTGAAAGT
CTACTATTTA GTAGTTTTCG AGGTAAATAA CATTGTGGTG GATAAAACTT TCAACTTTCA
       970        980        990       1000       1010       1020

AATGCGCGAA CAAGATTGAA GGTAATACCA ACAAGTAATC TATTCAATAA AAATGAGGAG
TTACGCGCTT GTTCTAACTT CCATTATGGT TGTTCATTAG ATAAGTTATT TTTACTCCTC
      1030       1040       1050       1060       1070       1080

TCTTTGTATT GGTTGTGTGT AAAAGGAGTC CCACCACTAA ATGATAATGA AAGCAATAAT
AGAAACATAA CCAACACACA TTTTCCTCAG GGTGGTGATT TACTATTACT TTCGTTATTA
      1090       1100       1110       1120       1130       1140

AAAAACAACA TAACTACGAA TCTTAATGTG AATGTGGTTA CGAATAGTTG TATTAAATTA
TTTTTGTTGT ATTGATGCTT AGAATTACAC TTACACCAAT GCTTATCAAC ATAATTTAAT
      1150       1160       1170       1180       1190       1200
```

ATTTATAGGC CTAAAACTAT AGACTTAACG ACAATGGAGA TTGCAGATAA ATTAAAGTTA
TAAATATCCG GATTTTGATA TCTGAATTGC TGTTACCTCT AACGTCTATT TAATTTCAAT
1210 1220 1220 1240 1250 1260

GAGAGAAAAG GAAATAGTAT AGTTATAAAG AATCCAACAT CATCATATGT GAATATTGCA
CTCTCTTTTC CTTTATCATA TCAATATTTC TTAGGTTGTA GTAGTATACA CTTATAACGT
1270 1280 1290 1300 1310 1320

AATATTAAAT CTGGTAATTT AAGTTTTAAT ATTCCAAATG GATATATTGA GCCATTTGGA
TTATAATTTA GACCATTAAA TTCAAAATTA TAAGGTTTAC CTATATAACT CGGTAAACCT
1330 1340 1350 1360 1370 1380

TATGCTCAAT TACCTGGTGG AGTACATAGT AAAATAACTT TGACTATTTT GGATGATAAC
ATACGAGTTA ATGGACCACC TCATGTATCA TTTTATTGAA ACTGATAAAA CCTACTATTG
1390 1400 1410 1420 1430 1440

GGCGCTGAAA TTATAAGAGA ATTATTAGTT TAAGGTGTAA AACAAATGAA GAAAACCACA
CCGCGACTTT AATATTCTCT TAATAATCAA ATTCCACATT TTGTTTACTT CTTTTGGTGT
1450 1460 1470 1480 1490 1500

ATTACTCTAT TTGTTTTAAC CAGTGTATTT CACTCTGGAA ATGTTTTCTC CAGACAATAT
TAATGAGATA AACAAAATTG GTCACATAAA GTGAGACCTT TACAAAAGAG GTCTGTTATA
1510 1520 1530 1540 1550 1560

AATTTCGACT ATGGAAGTTT GAGTCTTCTC CCGGTGAGAA TGCATCTTTT CTAAGTGTTG
TTAAAGCTGA TACCTTCAAA CTCAGAAGAG GGCCACTCTT ACGTAGAAAA GATTCACAAC
1570 1580 1590 1600 1610 1620

```
AAACGCTTCC CTGGTAATTA TGTTGTTGAT GTATATTTGA ATAATCAGTT AAAAGAAACT
TTTGCGAAGG GACCATTAAT ACAACAACTA CATATAAACT TATTAGTCAA TTTTCTTTGA
      1630       1640       1650       1660       1670       1680

ACTGAGTTGT ATTTCAAATC AATGACTCAG ACTCTAGAAC CATGCTTAAC AAAAGAAAAA
TGACTCAACA TAAAGTTTAG TTACTGAGTC TGAGATCTTG GTACGAATTG TTTTCTTTTT
      1690       1700       1710       1720       1730       1740

CTTATAAAGT ATGGGATCGC CATCCAGGAG CTTCATGGGT TGCAGTTTGA TAATGAACAA
GAATATTTCA TACCCTAGCG GTAGGTCCTC GAAGTACCCA ACGTCAAACT ATTACTTGTT
      1750       1760       1770       1780       1790       1800

TGCGTTCTCT TAGAGCATTC TCCTCTTTAA ATATACTTAT AACGCGGCTA ACCAAAGTTT
ACGCAAGAGA ATCTCGTAAG AGGAGAAATT TATATGAATA TTGCGCCGAT TGGTTTCAAA
      1810       1820       1830       1840       1850       1860

GCTTTTAAAT GCACCATCTA AAATTCTATC TCCAATAGAC AGTGAAATTG CTGATGAAAA
CGAAAATTTA CGTGGTAGAT TTTAAGATAG AGGTTATCTG TCACTTTAAC GACTACTTTT
      1870       1880       1890       1900       1910       1920

TATCTGGGAT GATGGCATTA ACGCTTTTCT TTTAAATTAC AGAGCTTAAT TATTTGCATT
ATAGACCCTA CTACCGTAAT TGCGAAAAGA AAATTTAATG TCTCGAATTA ATAAACGTAA
      1930       1940       1950       1960       1970       1980

CTAAGGTTGG AGGAGAGAGA TTCATACTTT GGTCAAATTC AACCTTGGTT TTAATTTTGG
GATTCCAACC TCCTCTCTCT AAGTATGAAA CCAGTTTAAG TTGGAACCAA AATTAAAACC
      1990       2000       2010       2020       2030       2040
```

```
TCCCTGGCGG CTAAGGAATC TATCATCTTG GCAAAACTTG TCAAGCGAAA AAAAATTTGA
AGGGACCGCC GATTCCTTAG ATAGTAGAAC AGTTTTGAAC AGTTCGCTTT TTTTTAAACT
      2050       2060       2070       2080       2090       2100

ATCAGCATAT ATTTATGCTG AGCGAGGTTT AAAAAAAATA AAGAGCAAAC TAACAGTTGG
TAGTCGTATA TAAATACGAC TCGCTCCAAA TTTTTTTTAT TTCTCGTTTG ATTGTCAACC
      2110       2120       2130       2140       2150       2160

GGACAAATAT ACCAGTGCAG ATTTATTCGA TAGCGTACCA TTTAGAGGCT TTTCTTTAAA
CCTGTTTATA TGGTCACGTC TAAATAAGCT ATCGCATGGT AAATCTCCGA AAAGAAATTT
      2170       2180       2190       2200       2210       2220

TAAAGATGAA AGTATGATAC CTTTCTCACA GAGAACATAT TATCCAACAA TACGTGGTAT
ATTTCTACTT TCATACTATG GAAAGAGTGT CTCTTGTATA ATAGGTTGTT ATGCACCATA
      2230       2240       2250       2260       2270       2280

TGCGAAAACC AATGCGACTG TAGAAGTAAG ACAAAATGGA TACTTGATAT ATTCTACTTC
ACGCTTTTGG TTACGCTGAC ATCTTCATTC TGTTTTACCT ATGAACTATA TAAGATGAAG
      2290       2300       2310       2320       2330       2340

                                            VI-3'
AGTCCCCCCC GGGCAATTCG AGATAGGTAG AGAACAAATT GCTGATC -3'
TCAGGGGGGG CCCGTTAAGC TCTATCCATC TCTTGTTTAA CGACTAG -5'
      2350       2360       2370       2380
```

CLAIMS

1. Recombinant DNA encoding for the Salmonella enteritidis fimbrial antigen amino acid sequence:

M L I V D F W R F C N M R K S A S A V A V L A L I A C G S A H A A G F
V G N K A E V Q A A V T I A A Q N T T S A N W S Q D P G F T G P A V A
A G Q K V G T L S I T A T G P H N S V S I A G K G A S V S G G V A T V
P F V D G Q G Q P V F R G R I Q G A N I N D Q A N T G I D G L A G W R
V A S S Q E T L N V P V T T F G K S T L P A G T F T A T F Y V Q Q Y Q
N

for an epitopic part thereof or for alleles of either.

2. Recombinant DNA as claimed in Claim 1 comprising the sequences I and II:

Sequence I

```
5'-  G CTCAGAATAC AACATCAGCC  AACTGGAGTC  AGGAT  -3'
3'-  C GAGTCTTATG TTGTAGTCGG  TTGACCTCAG  TCCTA  -5'
           230         240          250
```

Sequence II

```
5'-  CCTGG CTTTACAGGG CCTGCTGTTG CTGCTGGTCA GAAAGTTGGT
3'-  GGACC GAAATGTCCC GGACGACAAC GACGACCAGT CTTTCAACCA
         260        270        280        290        300

ACTCTCAGCA TTACTGCTAC TGGTCCACAT AACTCAGTAT CTATTGCAGG TAAAGGGGCT
TGAGAGTCGT AATGACGATG ACCAGGTGTA TTGAGTCATA GATAACGTCC ATTTCCCCGA
       310        320        330        340        350        360
```

TCGGTATCTG GTGGTGTAGC CACTGTCCCG TTCGTTGATG GACAAGGACA GCCTGTTTT -3'
AGCCATAGAC CACCACATCG GTGACAGGGC AAGCAACTAC CTGTTCCTGT CGGACAAAA -5'
370 380 390 400 410

sequences degenerately equivalent thereto, or sequences encoding for allelic variants of the part of SEFA for which the sequences I and II encode.

3. Recombinant DNA as claimed in Claim 1 or 2 wherein the sequence comprising sequences I and II comprises sequences III and IV:

Sequence III

5'- ATGCTAAT AGTTGATTTT TGGAGATTTT GTAATATGCG TAAATCAGCA
3'- TACGATTA TCAACTAAAA ACCTCTAAAA CATTATACGC ATTTAGTCGT
80 90 100 110 120

TCTGCAGTAG CAGTTCTTGC TTTAATTGCA TGTGGCAGTG CCCACGCAGC TGGCTTTGTT
AGACGTCATC GTCAAGAACG AAATTAACGT ACACCGTCAC GGGTGCGTCG ACCGAAACAA
130 140 150 160 170 180

GGTAACAAAG CAGAGGTTCA GGCAGCGGTT ACTATTGCAG CTCAGAATAC AACATCAGCC
CCATTGTTTC GTCTCCAAGT CCGTCGCCAA TGATAACGTC GAGTCTTATG TTGTAGTCGG
190 200 210 220 230 240

AACTGGAGTC AGGAT -3'
TTGACCTCAG TCCTA -5'
250

Sequence IV

```
5'-   CCTGG CTTTACAGGG CCTGCTGTTG CTGCTGGTCA GAAAGTTGGT
3'-   GGACC GAAATGTCCC GGACGACAAC GACGACCAGT CTTTCAACCA
        260        270        280        290        300

ACTCTCAGCA TTACTGCTAC TGGTCCACAT AACTCAGTAT CTATTGCAGG TAAAGGGGCT
TGAGAGTCGT AATGACGATG ACCAGGTGTA TTGAGTCATA GATAACGTCC ATTTCCCCGA
       310        320        330        340        350        360

TCGGTATCTG GTGGTGTAGC CACTGTCCCG TTCGTTGATG GACAAGGACA GCCTGTTTTC
AGCCATAGAC CACCACATCG GTGACAGGGC AAGCAACTAC CTGTTCCTGT CGGACAAAAG
       370        380        390        400        410        420

CGTGGGCGTA TTCAGGGAGC CAATATTAAT GACCAAGCAA ATACTGGAAT TGACGGGCTT
GCACCCGCAT AAGTCCCTCG GTTATAATTA CTGGTTCGTT TATGACCTTA ACTGCCCGAA
       430        440        450        460        470        480

GCAGGTTGGC GAGTTGCCAG CTCTCAAGAA ACGCTAAATG TCCCTGTCAC AACCTTTGGT
CGTCCAACCG CTCAACGGTC GAGAGTTCTT TGCGATTTAC AGGGACAGTG TTGGAAACCA
       490        500        510        520        530        540

AAATCGACCC TGCCAGCAGG TACTTTCACT GCGACCTTCT ACGTTCAGCA GTATCAAAAC -3'
TTTAGCTGGG ACGGTCGTCC ATGAAAGTGA CGCTGGAAGA TGCAAGTCGT CATAGTTTTG -5'
       550        560        570        580        590        600
```

sequences degenerately equivalent thereto or sequences which encode for allelic variants of SEFA.

4. Recombinant DNA as claimed in any one of the preceding claims wherein suitable flanking sequences for control of amino acid sequence expression are provided.

5. Recombinant DNA as claimed in any one of the preceding claims wherein the sequences I and II are provided in sequences comprising sequences V and VI respectively:

Sequence V

```
5'- GATCCTTGTT TTTTTTCTTA AATTTTTAAA ATGGCGTGAG TATATTAGCA TCCGCACAGA
3'- CTAGGAACAA AAAAAAGAAT TTAAAAATTT TACCGCACTC ATATAATCGT AGGCGTGTCT
            10         20         30         40         50         60

    TAAATTGTGC GAATGCTAAT AGTTGATTTT TGGAGATTTT GTAATATGCG TAAATCAGCA
    ATTTAACACG CTTACGATTA TCAACTAAAA ACCTCTAAAA CATTATACGC ATTTAGTCGT
            70         80         90        100        110        120

    TCTGCAGTAG CAGTTCTTGC TTTAATTGCA TGTGGCAGTG CCCACGCAGC TGGCTTTGTT
    AGACGTCATC GTCAAGAACG AAATTAACGT ACACCGTCAC GGGTGCGTCG ACCGAAACAA
           130        140        150        160        170        180

    GGTAACAAAG CAGAGGTTCA GGCAGCGGTT ACTATTGCAG CTCAGAATAC AACATCAGCC
    CCATTGTTTC GTCTCCAAGT CCGTCGCCAA TGATAACGTC GAGTCTTATG TTGTAGTCGG
           190        200        210        220        230        240

    AACTGGAGTC AGGAT -3'
    TTGACCTCAG TCCTA -5'
           250
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Sequence VI

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     5'- CCTGG CTTTACAGGG CCTGCTGTTG CTGCTGGTCA GAAAGTTGGT
     3'- GGACC GAAATGTCCC GGACGACAAC GACGACCAGT CTTTCAACCA
                260        270        280        290        300

ACTCTCAGCA TTACTGCTAC TGGTCCACAT AACTCAGTAT CTATTGCAGG TAAAGGGGCT
TGAGAGTCGT AATGACGATG ACCAGGTGTA TTGAGTCATA GATAACGTCC ATTTCCCCGA
       310        320        330        340        350        360

TCGGTATCTG GTGGTGTAGC CACTGTCCCG TTCGTTGATG GACAAGGACA GCCTGTTTTC
AGCCATAGAC CACCACATCG GTGACAGGGC AAGCAACTAC CTGTTCCTGT CGGACAAAAG
       370        380        390        400        410        420

CGTGGGCGTA TTCAGGGAGC CAATATTAAT GACCAAGCAA ATACTGGAAT TGACGGGCTT
GCACCCGCAT AAGTCCCTCG GTTATAATTA CTGGTTCGTT TATGACCTTA ACTGCCCGAA
       430        440        450        460        470        480

GCAGGTTGGC GAGTTGCCAG CTCTCAAGAA ACGCTAAATG TCCCTGTCAC AACCTTTGGT
CGTCCAACCG CTCAACGGTC GAGAGTTCTT TGCGATTTAC AGGGACAGTG TTGGAAACCA
       490        500        510        520        530        540

AAATCGACCC TGCCAGCAGG TACTTTCACT GCGACCTTCT ACGTTCAGCA GTATCAAAAC
TTTAGCTGGG ACGGTCGTCC ATGAAAGTGA CGCTGGAAGA TGCAAGTCGT CATAGTTTTG
       550        560        570        580        590        600

TAATTTAATT TAAACTTTAT AAATGCCCTC AATATGAGCG AGTTTGGATA ATTTTATTAT
ATTAAATTAA ATTTGAAATA TTTACGGGAG TTATACTCGC TCAAACCTAT TAAAATAATA
       610        620        630        640        650        660
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TTTAAAAATA TCTATTTTGA ATAGATAGGT TTTATGCTTC CATGCAAAAA CTTAAAGAGG
AAATTTTTAT AGATAAAACT TATCTATCCA AAATACGAAG GTACGTTTTT GAATTTCTCC
       670        680        690        700        710        720

GATTATGTAT ATTTTGAATA AATTTATACG TAGAACTGTT ATCTTTTTCC TTTTTTTTGC
CTAATACATA TAAAACTTAT TTAAATATGC ATCTTGACAA TAGAAAAAGG AAAAAAAACG
       730        740        750        760        770        780

TACCTTCCAA TTGCTTCTTC GGAAAGTAAA AAAATTGAGC AACCATTATT AACACAAAAA
ATGGAAGGTT AACGAAGAAG CCTTTCATTT TTTTAACTCG TTGGTAATAA TTGTGTTTTT
       790        800        810        820        830        840

TATTATGGCC TAAGATTGGG CACTACACGT GTTATTTATA AAGAAGATGC TCCATCAACA
ATAATACCGG ATTCTAACCC GTGATGTGCA CAATAAATAT TTCTTCTACG AGGTAGTTGT
       850        860        870        880        890        900

AGTTTTTGGA TTATGAATGA AAAAGAATAT CCAATCCTTG TTCAAACTCA AGTATATAAT
TCAAAAACCT AATACTTACT TTTTCTTATA GGTTAGGAAC AAGTTTGAGT TCATATATTA
       910        920        930        940        950        960

GATGATAAAT CATCAAAAGC TCCATTTATT GTAACACCAC CTATTTTGAA AGTTGAAAGT
CTACTATTTA GTAGTTTTCG AGGTAAATAA CATTGTGGTG GATAAAACTT TCAACTTTCA
       970        980        990       1000       1010       1020

AATGCGCGAA CAAGATTGAA GGTAATACCA ACAAGTAATC TATTCAATAA AAATGAGGAG
TTACGCGCTT GTTCTAACTT CCATTATGGT TGTTCATTAG ATAAGTTATT TTTACTCCTC
      1030       1040       1050       1060       1070       1080
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TCTTTGTATT GGTTGTGTGT AAAAGGAGTC CCACCACTAA ATGATAATGA AAGCAATAAT
AGAAACATAA CCAACACACA TTTTCCTCAG GGTGGTGATT TACTATTACT TTCGTTATTA
      1090       1100       1110       1120       1130       1140

AAAAACAACA TAACTACGAA TCTTAATGTG AATGTGGTTA CGAATAGTTG TATTAAATTA
TTTTTGTTGT ATTGATGCTT AGAATTACAC TTACACCAAT GCTTATCAAC ATAATTTAAT
      1150       1160       1170       1180       1190       1200

ATTTATAGGC CTAAAACTAT AGACTTAACG ACAATGGAGA TTGCAGATAA ATTAAAGTTA
TAAATATCCG GATTTTGATA TCTGAATTGC TGTTACCTCT AACGTCTATT TAATTTCAAT
      1210       1220       1220       1240       1250       1260

GAGAGAAAAG GAAATAGTAT AGTTATAAAG AATCCAACAT CATCATATGT GAATATTGCA
CTCTCTTTTC CTTTATCATA TCAATATTTC TTAGGTTGTA GTAGTATACA CTTATAACGT
      1270       1280       1290       1300       1310       1320

AATATTAAAT CTGGTAATTT AAGTTTTAAT ATTCCAAATG GATATATTGA GCCATTTGGA
TTATAATTTA GACCATTAAA TTCAAAATTA TAAGGTTTAC CTATATAACT CGGTAAACCT
      1330       1340       1350       1360       1370       1380

TATGCTCAAT TACCTGGTGG AGTACATAGT AAAATAACTT TGACTATTTT GGATGATAAC
ATACGAGTTA ATGGACCACC TCATGTATCA TTTTATTGAA ACTGATAAAA CCTACTATTG
      1390       1400       1410       1020       1430       1440

GGCGCTGAAA TTATAAGAGA ATTATTAGTT TAAGGTGTAA AACAAATGAA GAAAACCACA
CCGCGACTTT AATATTCTCT TAATAATCAA ATTCCACATT TTGTTTACTT CTTTTGGTGT
      1450       1460       1470       1480       1490       1500
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ATTACTCTAT TTGTTTTAAC CAGTGTATTT CACTCTGGAA ATGTTTTCTC CAGACAATAT
TAATGAGATA AACAAAATTG GTCACATAAA GTGAGACCTT TACAAAAGAG GTCTGTTATA
      1510       1520       1530       1540       1550       1560

AATTTCGACT ATGGAAGTTT GAGTCTTCTC CCGGTGAGAA TGCATCTTTT CTAAGTGTTG
TTAAAGCTGA TACCTTCAAA CTCAGAAGAG GGCCACTCTT ACGTAGAAAA GATTCACAAC
      1570       1580       1590       1600       1610       1620

AAACGCTTCC CTGGTAATTA TGTTGTTGAT GTATATTTGA ATAATCAGTT AAAAGAAACT
TTTGCGAAGG GACCATTAAT ACAACAACTA CATATAAACT TATTAGTCAA TTTTCTTTGA
      1630       1640       1650       1660       1670       1680

ACTGAGTTGT ATTTCAAATC AATGACTCAG ACTCTAGAAC CATGCTTAAC AAAAGAAAAA
TGACTCAACA TAAAGTTTAG TTACTGAGTC TGAGATCTTG GTACGAATTG TTTTCTTTTT
      1690       1700       1710       1720       1730       1740

CTTATAAAGT ATGGGATCGC CATCCAGGAG CTTCATGGGT TGCAGTTTGA TAATGAACAA
GAATATTTCA TACCCTAGCG GTAGGTCCTC GAAGTACCCA ACGTCAAACT ATTACTTGTT
      1750       1760       1770       1780       1790       1800

TGCGTTCTCT TAGAGCATTC TCCTCTTTAA ATATACTTAT AACGCGGCTA ACCAAAGTTT
ACGCAAGAGA ATCTCGTAAG AGGAGAAATT TATATGAATA TTGCGCCGAT TGGTTTCAAA
      1810       1820       1830       1840       1850       1860

GCTTTTAAAT GCACCATCTA AAATTCTATC TCCAATAGAC AGTGAAATTG CTGATGAAAA
CGAAAATTTA CGTGGTAGAT TTTAAGATAG AGGTTATCTG TCACTTTAAC GACTACTTTT
      1870       1880       1890       1900       1910       1920
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TATCTGGGAT GATGGCATTA ACGCTTTTCT TTTAAATTAC AGAGCTTAAT TATTTGCATT
ATAGACCCTA CTACCGTAAT TGCGAAAAGA AAATTTAATG TCTCGAATTA ATAAACGTAA
      1930       1940       1950       1960       1970       1980

CTAAGGTTGG AGGAGAGAGA TTCATACTTT GGTCAAATTC AACCTTGGTT TTAATTTTGG
GATTCCAACC TCCTCTCTCT AAGTATGAAA CCAGTTTAAG TTGGAACCAA AATTAAAACC
      1990       2000       2010       2020       2030       2040

TCCCTGGCGG CTAAGGAATC TATCATCTTG GCAAAACTTG TCAAGCGAAA AAAAATTTGA
AGGGACCGCC GATTCCTTAG ATAGTAGAAC AGTTTTGAAC AGTTCGCTTT TTTTTAAACT
      2050       2060       2070       2080       2090       2100

ATCAGCATAT ATTTATGCTG AGCGAGGTTT AAAAAAAATA AAGAGCAAAC TAACAGTTGG
TAGTCGTATA TAAATACGAC TCGCTCCAAA TTTTTTTTAT TTCTCGTTTG ATTGTCAACC
      2110       2120       2130       2140       2150       2160

GGACAAATAT ACCAGTGCAG ATTTATTCGA TAGCGTACCA TTTAGAGGCT TTTCTTTAAA
CCTGTTTATA TGGTCACGTC TAAATAAGCT ATCGCATGGT AAATCTCCGA AAAGAAATTT
      2170       2180       2190       2200       2210       2220

TAAAGATGAA AGTATGATAC CTTTCTCACA GAGAACATAT TATCCAACAA TACGTGGTAT
ATTTCTACTT TCATACTATG GAAAGAGTGT CTCTTGTATA ATAGGTTGTT ATGCACCATA
      2230       2240       2250       2260       2270       2280

TGCGAAAACC AATGCGACTG TAGAAGTAAG ACAAAATGGA TACTTGATAT ATTCTACTTC
ACGCTTTTGG TTACGCTGAC ATCTTCATTC TGTTTTACCT ATGAACTATA TAAGATGAAG
      2290       2300       2310       2320       2330       2340
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AGTCCCCCCC GGGCAATTCG AGATAGGTAG AGAACAAATT GCTGATC -3'
TCAGGGGGGG CCCGTTAAGC TCTATCCATC TCTTGTTTAA CGACTAG -5'
      2350      2360      2370      2380
```

or sequences degeneratively equivalent thereto.

6. Recombinant DNA as claimed in Claim 2 wherein the sequences I and II are comprised within a contiguous sequence VII (as described herein).

7. Recombinant DNA as claimed in Claim 3 wherein the sequences III and IV are comprised within a contiguous sequence VIII (as described herein).

8. Recombinant DNA as claimed in Claim 5 wherein the sequences V and VI are comprised within a contiguous sequence IX (as described herein).

9. Recombinant DNA as claimed in Claim 3 or Claim 7 wherein the amino acid sequence encoded is all or part of an allele of SEFA.

10. Recombinant DNA as claimed in any one of claims 1 to 5 further comprising a sequence encoding for a further amino acid sequence.

11. Recombinant DNA as claimed in Claim 10 wherein the further amino acid sequence comprises additional SEFA antigen or epitopic parts thereof.

12. Recombinant DNA as claimed in Claim 10 wherein the further amino acid sequence comprises a non-SEFA epitopic sequence.

13. Recombinant DNA as claimed in Claim 12 wherein the non-SEFA epitopic sequence comprises SB10 epitope of Mycobacterium bovis.

14. A novel plasmid comprising recombinant DNA as claimed in any one of Claims 1 to 13.

15. A plasmid as claimed in Claim 14 comprising a plasmid suitable for transformation of E.coli or yeast into which the recombinant DNA has been inserted.

16. A plasmid as claimed in Claim 14 or 15 comprising pBR322, pACYC184 or pUC18 into which the recombinant DNA has been inserted.

17. A transformant microorganism comprising a plasmid as claimed in any one of claims 14, 15 or 16.

18. A microorganism as claimed in Claim 17 wherein the plasmid host is a yeast or an E.coli.

19. A microorganism as claimed in Claim 18 wherein the plasmid host is an E. coli DH5alpha.

20. A plasmid as claimed in any one of Claims 14, 15 or 16 wherein the recombinant DNA sequences are produced by extracting total genomic DNA from an S. enteritidis or a SEFA expressing S. dublin; partially digesting the genomic DNA with SauIIIA restriction endonuclease to provide fragments in the size range 5 to 10 kilobases; ligating the fragments into a plasmid pBR322, pACYC184 or pUC18 and selecting desired plasmids for their ability to express SEFA, a part thereof or an allele of either.

21. A plasmid as claimed in Claim 20 wherein a further DNA sequence has been ligated into the BamH1 site in sequence I, III, V, VII, VIII or IX.

22. A plasmid as claimed in Claim 20 wherein the further DNA sequence is in frame with the SEFA expressing sequence.

23. A transformant microrganism as claimed in any one of Claims 17 to 19 wherein the plasmid is that as claimed in any one of Claims 20 to 22.

24. A polypeptide or oligopeptide comprising SEFA, an epitopic part thereof or alleles of either as expressed by a transformant as claimed in any one of Claims 17, 18, 19 or 23.

25. A test kit for the identification of microorganisms as being of either serotype S. enteritidis or S. dublin comprising a polypeptide as claimed in Claim 24.

26. A method for the determination of the presence of microorganisms having DNA or RNA polynucleotide sequence encoding for SEFA, an epitopic part thereof or alleles of either, or such DNA or RNA itself, comprising:

(a) providing a sample suspected of containing said encoding polynucleotide sequence;

(b) determining the presence of said sequence by monitoring hybridization of SEFA sequence targeted polynucleotide hybridization probes with said DNA or RNA.

27. A method as claimed in Claim 26 wherein the polynucleotide probes are targeted to any one of the sequences VII, VIII or XI.

28. A method as claimed in Claim 27 wherein the polynucleotide probe consists of sequence VII, VIII or XI.

29. A method for determining the presence of microorganisms having DNA or RNA polynucleotide sequence encoding for SEFA or an epitopic part thereof, or such DNA or RNA itself, comprising:

(a) providing a sample suspected of containing said encoding polynucleotide sequence;

(b) subjecting said sample to conditions under which polynucleotide sequences comprising sequences (I) and (II) are replicated by use of the polymerase chain reaction;

(c) determining the presence of any sequence produced.

30. A method as claimed in Claim 29 wherein the step (c) is carried out using a polynucleotide hybridization probe.

31. A method as claimed in either of Claim 29 or Claim 30 wherein step (b) employs primer pairs comprising one primer selected from group (A) and the other from group (B):

Group A:	Group B:
5' -GTGCGAATGCTAATAGTTGA- 3'	5' -AAAACAGGCTGTCCTTGTCCA- 3'
5' -TGCGTAAATCAGCATCTGCA- 3'	5' -TTAGCGTTTCTTGAGAGCTGG- 3'
5' -TCTGCAGTAGCAGTTCTTGC- 3'	5' -TTTTGATACTGCTGAACGTAG- 3'
5' -GCTCAGAATACAACATCAGCCAA- 3'	

32. A method as claimed in any one of Claims 29 to 31 wherein the step (c) is carried out using an oligonucleotide probe selected from sequences of either of groups A or B (as described herein) which is different to that of either of the primers used for step (b).

33. A test kit for performing the method of any one of Claims 26 to 28 comprising polynucleotide hybridization probes targeted at sequence VII, VIII.

34. A test kit as claimed in Claim 33 wherein the probes comprise sequences comprising sequence VII or VIII.

35. A test kit for performing the method of any one of Claims 29 to 32 comprising primers and probes having sequences selected from the groups (A) and (B).

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01691

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/31; C12N15/62; C12Q1/68; G01N33/569; C07H21/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N ; C12Q ; G01N ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	JOURNAL OF BACTERIOLOGY vol. 170, no. 9, September 1988, AMERICAN SOCIETY FOR MICROBIOLOGY pages 4216 - 4222; FEUTRIER, J. ET AL.: 'Cloning and expression of a Salmonella enteritidis fimbrin gene in Escherichia coli' see the whole document ---	1-9, 14-15, 17-18, 24,26-27
X	JOURNAL OF BACTERIOLOGY vol. 168, no. 1, October 1986, AMERICAN SOCIETY FOR MICROBIOLOGY pages 221 - 227; FEUTRIER, J. ET AL.: 'Purification and characterization of fimbriae from Salmonella enteritidis'	1,9,11, 24,26-27
Y		10,12-23
A	see the whole document ---	2-8,25, 28-35
	-/--	

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
06 JANUARY 1992	16 JAN 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	ANDRES S.M.

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	WO,A,8 910 967 (PRAXIS BIOLOGICS, INC.) 16 November 1989 see the whole document ---	10,12-23
A	JOURNAL OF GENERAL MICROBIOLOGY vol. 136, no. 2, February 1990, COLCHESTER, GB pages 265 - 272; RADFORD, A.J. ET AL.: 'Epitope mapping of the Mycobacterium bovis secretory protein MPB70 using overlapping peptide analysis' cited in the application see the whole document ---	10,12-23
A	EP,A,0 383 509 (ORTHO DIAGNOSTIC SYSTEMS, INC.) 22 August 1990 see the whole document ---	26-35

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9101691 SA 51809

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 06/01/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8910967	16-11-89	AU-A-	3697989	29-11-89
		EP-A-	0419513	03-04-91
EP-A-0383509	22-08-90	CA-A-	2009708	13-08-90
		JP-A-	2295498	06-12-90

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